

## PROTOCOL FOR Th-IRES-Cre MOUSE GENOTYPING

### *Procedure*

Genotyping of offspring from Th-IRES-Cre breeding colony is based on PCR.

### *PCR primers*

5' forward primer (Cre-UD): 5' gat acc tgg cct ggt ctg 3'  
3' reverse primer (TH-5'): 5' cac cct gac cca agc act 3'  
(TH-3'): 5' ctt tcc ttc ctt tat tga gat 3'

### *PCR profile – TEDD*

94 °C, 5 min

94 °C, 45 s                      35 cycles

56 °C, 45 s

72 °C, 1 min

72 °C, 10 min

4 °C, ∞

### *PCR mix*

10 x PCR Gold buffer (Perkin Elmer)	3.0 µl
MgCl <sub>2</sub> (25 mM)	2.5 µl
dNTPs (10 mM)	0.5 µl
Cre-UD (20 µM)	0.5 µl
TH-3' (20 µM)	0.5 µl
TH-5' (20 µM)	0.5 µl
AmpliTaq Gold (5 U/µl)	0.2 µl
DNA template (~ 0.5 µg tail DNA)	2.0 µl
ddH <sub>2</sub> O	<u>20.3 µl</u>
	30 µl

### *Post-PCR analysis*

Load 10 µl of the PCR reaction on a 1,5 % agarose gel.  
Expected results; two bands – 290 bp and 430 bp.